The prevalence of *Rickettsia felis* DNA in fleas collected from cats and dogs in the UK

[1,4]Swaid Abdullah, [2]Phillipa Lait, [2]Chris Helps, [3]Hannah Newbury, [1*]Richard Wall

[1]*Veterinary Parasitology and Ecology Group, School of Biological Sciences, University of Bristol, Bristol, UK*

[2]*Molecular Diagnostic Unit, Langford Vets, University of Bristol, Bristol, UK*

[3]*MSD Animal Health, Walton Manor, Walton, Milton Keynes, UK*

[4]*School of Veterinary Science, University of Queensland, Australia*

ABSTRACT

In a large-scale survey in the UK, recruited veterinary practices were asked to inspect client-owned cats and dogs, selected at random between April and June 2018, following a standardised flea inspection protocol. A total of 326 veterinary practices participated and 812 cats and 662 dogs were examined during the 3-month period. Fleas were collected, identified to species level and fleas of the same species collected from a single animal were pooled together and treated as a single sample. A total of 470 pooled flea samples were screened by PCR and DNA sequence analysis for a subset of *Rickettsia* species including *R. felis* and *R. typhi*. On analysis, 27 (5.7%) of the pooled flea samples were positive for *R. felis* DNA; these were predominantly in the cat flea, *Ctenocephalides felis*, but one dog flea, *Ctenocephalides canis* was also positive for this pathogen.

Key words: Emerging Disease, PCR, Pet, Siphonaptera, Surveillance, Vector, Zoonosis.

*Corresponding author.

E-mail address: richard.wall@bristol.ac.uk

1. Introduction

Rickettsia felis is an emerging bacterial pathogen and the aetiological agent responsible for flea-borne spotted fever (also known as cat flea typhus), which affects a range of vertebrates, including humans (Parola, 2011). It is now found worldwide in association with its primary vector and reservoir, the cat flea *Ctenocephalides felis*. Knowledge of *R. felis* remains relatively limited, particularly in relation to its epidemiology (Brown & Macaluso, 2016). Infection is transferred between flea life cycle stages transtadially and maintained within flea populations by transovarial transmission, although horizontal amplification within an infected host may be required for long-term maintenance in co-feeding flea populations. *Rickettsia felis* has been detected in a wide range of arthropods, including other flea species, ticks, mites and mosquitoes, although the role of these as vectors is unclear (Reif and Macaluso, 2009). In some regions, there is evidence of a high prevalence of *R. felis* in dogs, which might indicate a role of the canine host as a reservoir (Hii et al., 2013; Horta et al., 2014). A serological survey of 286 healthy cats in central Italy found 23 (8.04%) and 18 (6.29%) cats positive for *R. felis* and *R. conorii*, respectively (Morganti et al., 2019). Reports from Germany and Spain, where dog owners were suffering from flea-borne spotted fever, showed that their dogs tested positive for the pathogen even though the animals were broadly asymptomatic (Richter et al. 2002; Oteo et al. 2006). Further, a study on seroprevalence of *R. felis* in dogs in Spain indicated that 51.1% were seropositive for this pathogen (Nogueras et al., 2009).

Although cats are thought to be the primary reservoir for *R. felis* (Higgins et al. 1996, Gerhold et al., 2013), infection with *R. felis* causes little clinical disease (Barrs et al., 2010). A small percentage of infected cats may show clinical signs such as fever, although this is rare; immune-mediated thrombocytopenia, a disorder of red blood cells resulting in a low platelet count, may also be associated with infection (Wedincamp and Foil, 2000). Feline rickettsial infection in Europe, North and South America, Africa, Australia and Asia has been detected in serological studies (Case et al., 2006, Bayliss et al., 2009). However, no *R. felis* was found in studies conducted in the United States (Bayliss et al., 2009) and Canada (Kamrani et al., 2008) using *gltA* and/or *ompB* gene amplification in high-risk groups of cats.

Given the indiscriminate feeding habits of cat fleas (Azad et al., 1992), the zoonotic risk from *R. felis* may be high, especially where companion animals live in close contact with humans. As a result, it has been proposed that *R. felis* should be classified as an emerging global threat to human health (Yazid Abdad et al., 2011). In humans, infection results in a serious debilitating illness, with high fever, local lymphadenopathy, headaches, neurological signs, myalgia, and often

a maculopapular rash (Pérez-Osorio et al., 2008; Nilsson et al., 2014). Usefully, domestic pets can act as sentinels for such vector-borne zoonoses (Richter et al., 2002; Oteo et al., 2006). The current study examined the prevalence of *R. felis* in fleas collected in a randomised sample from cats and dogs in the UK to help quantify the risk of flea-borne *R. felis* infection in companion animals and humans in shared spaces.

2. Methods

2.1 Flea samples

The flea samples used in the current study were collected from both cats and dogs by veterinary surgeons throughout the UK as part of a national surveillance study; sampling details have been published previously (Abdullah et al., 2019). Enrolled veterinary practitioners selected 5 cats and 5 dogs per week at random for four weeks and undertook a standardised flea inspection using a dampened comb. At the end of the grooming process, the entire comb was placed in a plastic sample bag, sealed and sent by standard post to the University of Bristol where they were stored at −20 °C. Veterinarians were asked to complete a case history for each animal regardless of whether or not fleas were found. Identification of fleas was performed with the use of light microscopy and taxonomic keys (Whitaker, 2007).

After identification, fleas were transferred into individual micro-tubes and all the fleas of the same species collected from a single animal were pooled. DNA was extracted from each pooled flea sample using a high-throughput system, DNeasy 96 Blood & Tissue Kit (QIAGEN®, Manchester, UK). The flea samples were crushed using micro-pestles in their respective tubes and thoroughly mixed in 180 µl Buffer ATL and 20 µl proteinase K by vortexing. The samples were briefly centrifuged (2900 xg for 120 s) and incubated overnight at 56 °C to ensure complete tissue lysis. After overnight incubation, the lysates were briefly centrifuged (2900 xg for 120 s) and the liquid from each tube was transferred to an individual well of a 96 deep-well plate, leaving behind the flea exoskeleton. Further extraction steps were carried out as per the manufacturer's guidelines.

Flea DNA in the extracted samples was detected with conventional PCR that amplified a 1200 base pair (bp) region of the flea 18S rRNA gene. A master mix was made as follows: 5 µl of 2 x GoTaq Hot Start Mastermix (Promega, UK), 0.2 µl of 10 µM each forward (18S-F)/reverse (18S-R) primes and 2.8 µl water. A high-throughput automated pipetting system (epMotion P5073, Eppendorf, Stevenage, UK) was used to add 2 µl of flea DNA to 8 µl of master mix in 96 well PCR plates Flea DNA and water were used as positive and negative controls,

respectively. The thermal cycling protocol consisted of an initial denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 20 s, 56 °C for 20 s and 72 °C for 90 s in a thermal cycler (Biorad T100 thermal cycler, Biorad, Watford, UK). Amplified DNA was subjected to electrophoresis in a 1.5% agarose gel pre-stained with 0.05 μg/ml ethidium bromide and viewed under ultraviolet light. Positive samples were identified as having a defined band of ~1200 bp on the gel.

2.2 Rickettsia spp. quantitative PCRs and DNA sequencing

Flea DNA samples were screened for a subset of *Rickettsia* species including *R. felis* and *R. typhi* as primary flea borne *Rickettsia* (Lucas et al., 2017), using real-time PCR primers designed to amplify a 147 bp fragment of the citrate synthase gene (*gltA*). Resulting forward 5'-AGGGTCTTCGTGCATTTCTT-3' and reverse 5'-GAGAGAAAATTATATCCAAATGTTGAT-3' primers (modified from Labruna et al 2004) were combined with a fluorogenic probe 5' 6-FAM-CACTGTGCCATCCAGCCTACGGT-BHQ-1 3'). The PCR comprised 12.5 μL 2 x GoTaq Hot Start Mastermix (Promega, UK), 0.5 μL of 10 uM each forward and reverse primers, 0.25 μL 10uM probe, 1.5 μL 50 nM $MgCl_2$, 10 μL water and 5 μL DNA. Amplification was performed in a Statagene Mx3005P QPCR system for 2 min at 95 °C followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Negative control (molecular grade water) and positive control (*R. felis* DNA received from Australian Rickettsial Reference Laboratory, Barwon Health Geelong, VIC 3220, Australia) were included. The assay was optimised by using a series of dilutions of *R. felis* positive controls and estimation of reaction efficiency. PCR products from positives were cleaned up directly using the Nucleopsin Gel and PCR Clean-up kit (Machery-Nagel, Düren, Germany) according to manufacturer's instructions. DNA sequencing was performed by DNA Sequencing and Services (MRC I PPU, School of Life Sciences, University of Dundee, Scotland, www.dnaseq.co.uk) using Applied Biosystems Big-Dye Ver 3.1 chemistry on an applied Biosystems model 3730 automated capillary DNA sequencer. Similarity to published sequences was determined using BLAST (http//www.ncbi.nlm.nih.gov) hosted by the National Centre for Biotechnology Information.

3. Results and Discussion

A total of 326 veterinary practices from across the UK participated in the survey between April and June 2018, and a total of 1,627 animals were examined. For 1,475 of these animals a

wholly, or at least partially, completed case history was submitted and consisted of 812 cats, 662 dogs and one animal of unspecified species. No case history details were submitted for 152 samples. The flea infestation rate was high for both cats (28.1% ±3.1%) and dogs (14.4%±2.7%), and the results from this survey have been described and discussed in detail by Abdullah et al. (2019).

A total of 470 pooled flea samples collected from 94 dogs, 227 cats and 149 with no host record, were analysed for the presence of two flea borne *Rickettsia* spp. Of these 470 samples, 429 were cat flea samples (*C. felis felis*), 9 dog flea samples (*C. canis*), 6 hedgehog flea samples (*Archaeopsylla erinacei*), 19 rabbit flea samples (*Spilopsyllus cuniculi)* and 7 chicken flea samples (*Ceratophyllus* spp.). On PCR analysis, a total of 38 flea samples were positive for *Rickettsia* species DNA, and on sequencing 27 of these positive samples were found to be *R. felis* (19 had a full case history and 8 had no case history) as presented in Table 1, giving a prevalence of 5.7% for *R. felis*. Previous studies of the prevalence of *R. felis* in the UK also reported similar infection rates of 6% to 12% (Kenny et al. 2003, whereas Shaw et al. 2004 reported a much higher prevalence of 21%. However, both these studies were relatively localised and focussed their sampling across southern parts of the UK and Northern Ireland.

Of the remaining 11 flea samples that were positive by PCR assay, the analysis for sequence similarity using BLAST indicated 4 of the sequences match closely (98.8-100%) with *R. asembonensis*; 3 of these samples were from hedgehog fleas (*Archaeopsylla erinacei*) and one was from a cat flea. One sample was from a dog and one was from a cat, the other two had no records of the host species. *R. asembonensis* belongs to a group of *R. felis*–like organisms (RFLOs), which are closely related to *R. felis* (Jiang et al., 2013); to distinguish *R. asembonensis* from *R. felis*, further sequence analysis of additional genes such as *ompA*, *ompB* or *sca4* would be desirable (Maina et al., 2016) . This species is ubiquitous and has been reported from multiple ectoparasites (Oteo et al., 2014; Maina et al., 2016), but its pathogenic significance remains unknown. Among the remaining 7 PCR positive samples, two were found to carry the DNA of an unknown *Rickettsia* sp. and the remaining 5 samples did not produce a sequence that could be analysed.

In addition to cat fleas, *R. felis* DNA was also detected in one *C. canis* (dog flea), indicating that other flea species may also act as vectors of this pathogen, and other animal species may be potential reservoirs of infection. Similar findings were reported from Germany by Gilles et al. (2008), where they found that *A. erinacei* (hedgehog flea) carried *R. felis*, suggesting the hedgehog as a potential reservoir of infection. However, while the detection of pathogen DNA

in fleas may indicate concurrent infection of the host and the vector, since no blood samples were collected from the hosts in this study, the prevalence of *R. felis* infection in the host cannot be determined.

Rickettsia felis is an important emerging zoonosis worldwide (Parola et al., 2005; Pérez-Osorio et al., 2008; Teoh et al., 2017). In this study, a *R. felis* infection prevalence of 5.7% was detected in fleas collected from cats and dogs in the UK. Even though the prevalence of this pathogen in fleas may seem relatively low in comparison to some studies in central Europe, fleas are frequent feeders (Cadiergues et al., 2000) and their numbers can increase quickly under favourable conditions (Silverman et al. 1981), which can rapidly increase the risk of flea bites and the transmission of this pathogen. *Rickettsia felis* appears to be widely distributed within the UK, infecting a geographically dispersed population of cat fleas. In humans, infection causes symptoms that are similar to those of murine typhus and other febrile illnesses such as dengue, with fever and myalgia (Pérez-Osorio et al., 2008). Clinicians coming across patients with fever and/or rash should consider a differential diagnosis of *R. felis,* particularly if the patient is known to have been exposed to flea bites. Hence, the effective year-round flea control of fleas on pets and in the environment is important, both to reduce the direct effects of flea feeding and the risk of pathogen transmission.

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References

Abdullah, S., Helps, C., Tasker, S., Newbury, H., Wall, R. 2019. Pathogens in fleas collected from cats and dogs: distribution and prevalence in the UK. Parasites Vectors 12, 8. http://doi.org/10.1186/s13071-019-3326-x

193 Azad, A.F., Sacci, J.B., Nelson, W.M., Dasch, G.A., Schmidtmann, E. T., Carl, M. 1992. Genetic
194 characterization and transovarial transmission of a typhus-like *Rickettsia* found in cat fleas.
195 PNAS. 89, 43-46.

196 Barrs, V. R., Beatty, J. A., Wilson, B. J., Evans, N., Gowan, R., Baral, R. M., Lingard, A.E.,
197 Perkovic, G., Hawley, J.R., Lappin, M.R. 2010. Prevalence of *Bartonella* species, *Rickettsia*
198 *felis*, *haemoplasmas* and the *Ehrlichia* group in the blood of cats and fleas in eastern Australia.
199 Aust. Vet. J. 88, 160-165.

200 Bayliss, D. B., Morris, A. K., Horta, M. C., Labruna, M. B., Radecki, S. V., Hawley, J.R., Brewer,
201 M.M., Lappin, M. R. 2009. Prevalence of *Rickettsia* species antibodies and *Rickettsia* species
202 DNA in the blood of cats with and without fever. J. Feline Med. Surg. 11, 266-270.

203 Brown, L.D., Macaluso, K.R. 2016. *Rickettsia felis*, an emerging flea-borne rickettsiosis. Curr.
204 Trop. Med. Rep. 3, 27-39.

205 Cadiergues, M. C., Hourcq, P., Cantaloube, B., Franc, M. 2000. First bloodmeal of *Ctenocephalides*
206 *felis felis* (Siphonaptera: Pulicidae) on cats: time to initiation and duration of feeding. J. Med.
207 Entomol. 37, 634-636.

208 Higgins, J. A., Radulovic, S., Schriefer, M. E., Azad, A.F. (1996). *Rickettsia felis*: a new species of
209 pathogenic rickettsia isolated from cat fleas. J. Clin. Microbiol. 34, 671-674.

210 Hii, S. F., Abdad, M. Y., Kopp, S. R., Stenos, J., Rees, R.L., Traub, R. J. 2013. Seroprevalence
211 and risk factors for *Rickettsia felis* exposure in dogs from Southeast Queensland and the
212 Northern Territory, Australia. Parasites Vectors 6, 159. https://doi.org/10.1186/1756-
213 3305-6-159.

214 Gerhold, R. W., Jessup, D. A. 2013. Zoonotic diseases associated with free-roaming cats.
215 Zoonoses Public Hlth. 60, 189-195.

216 Gilles J, Thomas F, Hellmann JK, Silaghi C, Pradel I, Friche Passos LM, Lengauer H, Pfister K.
217 2008. *Rickettsia felis* in fleas, Germany. Emerg. Infect. Dis. 14, 1294–1296.

218 Horta, M. C., Ogrzewalska, M., Azevedo, M. C., Costa, F. B., Ferreira, F., Labruna, M. B. 2014.
219 *Rickettsia felis* in *Ctenocephalides felis felis* from five geographic regions of Brazil. Am. J. Trop.
220 Med. Hyg. 91, 96-100.

221 Jiang, J., Maina, A. N., Knobel, D. L., Cleaveland, S., Laudisoit, A., Wamburu, K., Ogola, E.,
222 Parola, P., Breiman, R.F., Kariuki Njenga, M., Richards, A.L. 2013. Molecular detection of

223 *Rickettsia felis* and *Candidatus Rickettsia asemboensis* in fleas from human habitats, Asembo,
224 Kenya. Vector-Borne Zoonot. 13, 550-558.

225 Kamrani, A., Parreira, V.R., Greenwood, J., Prescott, J.F. 2008. The prevalence of *Bartonella*,
226 hemoplasma, and *Rickettsia felis* infections in domestic cats and in cat fleas in Ontario. Can.
227 J. Vet. Res. 72, 411-419.

228 Kenny, M. J., Birtles, R. J., Day, M. J., Shaw, S. E. 2003. *Rickettsia felis* in the United Kingdom.
229 Emerg. Infect. Dis. 9, 1023–1024.

230 Blanton, L. S., Walker, D. H. 2017. Flea-borne rickettsioses and rickettsiae. Am. J. Trop. Med.
231 Hyg. 96, 53-56.

232 Maina, A. N., Luce-Fedrow, A., Omulo, S., Hang, J., Chan, T. C., Ade, F., Jima, D.D. Ogola, E.,
233 Ge, H., Breiman, R.F., Njenga, M.K. 2016. Isolation and characterization of a novel
234 *Rickettsia* species (*Rickettsia asembonensis* sp. nov.) obtained from cat fleas (*Ctenocephalides*
235 *felis*). Int. J. Syst. Evol. Micr. 66, 4512– 4517.

236 Morganti, G., Veronesi, F., Stefanetti, V., Di Muccio, T., Fiorentino, E., Diaferia, M., Santoro,
237 A., Passamonti, F., Gramiccia, M. 2019. Emerging feline vector-borne pathogens in Italy.
238 Parasites Vectors 12, 193. http://doi.org/10.1186/s13071-019-3409-8

239 Nilsson, K., Wallmenius, K., Hartwig, S., Norlander, T., Påhlson, C. 2014. Bell's palsy and
240 sudden deafness associated with *R. rickettsia* spp. infection in Sweden. A retrospective and
241 prospective serological survey including PCR findings. Eur. J. Neurol. 21, 206-214.

242 Nogueras, M. M., Pons, I., Ortuno, A., Lario, S., & Segura, F. 2011. *Rickettsia felis* in fleas from
243 Catalonia (Northeast Spain). Vector-Borne Zoonot. 11, 479-483.

244 Oteo, J. A., Portillo, A., Portero, F., Zavala-Castro, J., Venzal, J. M., Labruna, M.B. 2014.
245 *Candidatus Rickettsia asemboensis*' and *Wolbachia* spp. in *Ctenocephalides felis* and *Pulex irritans*
246 fleas removed from dogs in Ecuador. Parasites Vectors 7, 455.
247 http://doi.org/10.1186/s13071-014-0455-0

248 Oteo, J. A., Portillo, A., Santibáñez, S., Blanco, J. R., Pérez-Martínez, L., Ibarra, V. 2006. Cluster
249 of cases of human *Rickettsia felis* infection from Southern Europe (Spain) diagnosed by
250 PCR. J. Clin. Microbiol. 44, 2669-2671.

251 Parola, P., Davoust, B., Raoult, D. (2005). Tick-and flea-borne rickettsial emerging zoonoses.
252 Vet. Res. 36, 469-492.

253 Parola, P. 2011. *Rickettsia felis*: from a rare disease in the USA to a common cause of fever in sub-
254 Saharan Africa. Clin. Microbiol. Infect. 17, 996-1000.

255 Pérez-Osorio, C. E., Zavala-Velázquez, J. E., León, J. J. A., Zavala-Castro, J. E. 2008. *Rickettsia*
256 *felis* as emergent global threat for humans. Emerging Infect. Dis. 14, 1019–1023.

257 Reif, K. E., & Macaluso, K. R. 2009. Ecology of *Rickettsia felis*: a review. J. Med. Entomol. 46,
258 723-736.

259 Richter, J., Fournier, P. E., Petridou, J., Häussinger, D., Raoult, D. 2002. *Rickettsia felis* infection
260 acquired in Europe and documented by polymerase chain reaction. Emerging Infect. Dis.
261 8, 207-208.

262 Shaw, S. E., Kenny, M. J., Tasker, S., Birtles, R. J. 2004. Pathogen carriage by the cat flea
263 *Ctenocephalides felis* (Bouché) in the United Kingdom. Vet. Microbiol. 102, 183-188.

264 Silverman, J., Rust, M. K., Reierson, D. A. 1981. Influence of temperature and humidity on
265 survival and development of the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). J.
266 Med. Entomol. 18, 78-83.

267 Teoh, Y.T., Hii, S.F., Stevenson, M.A., Graves, S., Rees, R., Stenos, J., Traub, R.J. 2017.
268 Serological evidence of exposure to *Rickettsia felis* and *Rickettsia typhi* in Australian
269 veterinarians. Parasites Vectors 10, 129. http://doi.org/10.1186/s13071-017-2075-y

270 Whitaker, A.P. Siphonaptera. Handbooks for the Identification of British Insects. The Natural
271 History Museum, London. 2007; 1: 1-178.

272 Wedincamp, J. J., Foil, L. D. 2000. Infection and seroconversion of cats exposed to cat fleas
273 (*Ctenocephalides felis* Bouche) infected with *Rickettsia felis*. J. Vector Ecol. 25, 123-126.

274 Yazid Abdad, M., Stenos, J., Graves, S. 2011. *Rickettsia felis*, an emerging flea-transmitted human
275 pathogen. Emerg. Health Threats J. 7168. http://doi.org/10.3402/ehtj.v4i0.7168
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282 Table 1. The number of *Rickettsia felis*, *R. asembonensis* and 'other' *Rickettsia* positive samples that could not
283 be identified to species level, in flea samples of different species collected from cats and dogs (94 dogs,
284 227 cats and 149 with no host record) during a national survey in the UK.

285

Rickettsia species	Pet species	Number of Flea samples infected	*C. felis*	*C. canis*	Other Flea species
Rickettsia felis	Cat	14	13	1	
	Dog	5	5		
	No case history	8	8		
	Total	**27**	**26**	**1**	
Rickettsia asembonensis	Cat	4	1		3*
	Total	**4**	**1**		**3***
Unknown *Rickettsia*	Cat	1			1**
	No case history	1			
	Total	**2**			**1**

286 * *Archaeopsylla erinacei* ** *Spilopsyllus cuniculi*